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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

OPP OFFICIAL RECORD
HEALTH EFFECTS DIVISION
SCIENTIFIC DATA REVIEWS
EPA SERIES 361

MEMORANDUM

DATE: December 18, 2007

SUBJECT: Deltamethrin Toxicology Data Evaluation Record.
PC Code: 097805 DP Barcode: D333943 TXR #: 0054481

TO: Olga Oodiott/George Larocca, RM 13
Insecticide Branch
Registration Division

FROM: Karlyn J Bailey, Toxicologist
Registration Action Branch 2
Health Effects Division (7509 P)

THRU: Richard Loranger, Branch Senior Scientist
Registration Action Branch 2
Health Effects Division (7509 P)

for

ACTION REQUESTED: The Health Effects Division (HED) was asked to review the developmental neurotoxicity study conducted with Deltamethrin. The Data Evaluation Record (DER) is included for the MRID listed below in Table 1.

Table 1. Deltamethrin Toxicology Study

Study Type	MRID #	Studies included in review	Comments
870.6300 Developmental Neurotoxicity Study	46814301	N/A	New DER

Received in RPC 01/31/2008

DATA EVALUATION RECORD

DELTAMETHRIN

STUDY TYPE: DEVELOPMENTAL NEUROTOXICITY STUDY IN RATS (§83-6)
MRID 46814301

Prepared for

Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by

Toxicology and Hazard Assessment Group
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Work Assignment No. 164-2007

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory, managed and operated by UT-Battelle, LLC., for the U.S. Dept. of Energy under contract DE-AC05-00OR22725.

EPA Reviewer: Karlyn J. Bailey, M.S. **Signature:** _____
Registration Action Branch 2, Health Effects Division (7509P) **Date:** _____
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Health Effects Division (7509P) **Date:** _____

Template version 02/06

TXR#: 0054481

DATA EVALUATION RECORD

STUDY TYPE: Developmental Neurotoxicity Study - Rat;
OPPTS 870.6300 (' 83-6); OECD 426 (draft)

PC CODE: 097805

DP BARCODES: D333943

TEST MATERIAL (PURITY): Deltamethrin (98.8-99.6% a.i.)

SYNONYMS: (S)-Cyano-3-phenoxybenzyl(1R, 3R)-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropanecarboxylate

CITATION: Gilmore, R.G., L.P. Sheets, and H.E. Hoss (2006) A developmental neurotoxicity screening study with technical grade deltamethrin in Wistar rats. Bayer CropScience LP Toxicology, Stilwell, KS. Study number 04-D72-WO, April 3, 2006. MRID 46814301. Unpublished .

SPONSOR: Bayer CropScience, LP, Research Triangle Park, NC

EXECUTIVE SUMMARY: In a developmental neurotoxicity study (MRID 46814301), Deltamethrin (98.8-99.6 % a.i., batch 2350014) was administered in the diet to 30 female Wistar [HAN CRL:WI (GLX/BRL/HAN) IGSBR] rats per dose at dose levels of 0, 20, 80 or 200 ppm (average daily intake of 0, 1.64, 6.78 and 16.1 mg/kg/day, respectively) from gestation day (GD) 6 through lactation day (LD) 21. Because of increased food consumption during lactation, dietary levels were adjusted to achieve a more consistent mg/kg/day dosage throughout the exposure period. A Functional Observational Battery (FOB) was performed on all dams on GDs 13 and 20 and LDs 11 and 21. On postnatal day (PND) 4, litters were culled to yield four males and four females (as closely as possible). Offspring were allocated for detailed clinical observations (FOB) and assessment of motor activity, auditory startle reflex habituation, learning and memory (passive avoidance and water maze testing), and neuropathology at termination (PND 75±5). On PND 21, the whole brain was collected from10 pups/sex/group for micropathologic examination and morphometric analysis. Pup physical development was evaluated by body weight. The age of sexual maturation (vaginal opening in females and preputial separation in males) was assessed.

No deaths or clinical signs of toxicity were observed in parental females. During gestation, body weight was significantly decreased (6-7%) in females at 200 ppm on GDs 13 and 20. Body

weight gain for GDs 0-20 and food consumption for GDs 6-13 were significantly decreased (both 17%) for high-dose females. During lactation, body weight (6-8%) and food consumption (9%) were decreased in high-dose females on LD 0-7 but were comparable to the control group for the remainder of lactation.

No treatment-related effects on reproduction were reported. Litter size and viability were not affected by treatment. No clinical signs of toxicity were observed in offspring. Body weight was significantly decreased in male (7-10%) and female (6-9%) offspring at 200 ppm beginning on PND 4. The lower body weight was a result of decreased body weight gain during PNDs 1-4 and 4-11. For high-dose males, body weight gain during PNDs 1-4 and 4-11 was reduced by 15% and 10%, respectively, compared with the controls resulting in overall weight gain during lactation reduced by 8%. For high-dose females, body weight gain during PNDs 1-4 and 4-11 was reduced by 18% and 10%, respectively, compared with the controls resulting in overall weight gain during lactation reduced by 7%. During postweaning, the decreased body weight in the high-dose animals that developed during lactation persisted for one week in females (7%) and for four weeks in males (4-8%). The onset of balanopreputial separation was delayed in males at 200 ppm; however, this finding was attributed to the lower body weight of these animals compared with the controls.

On PND 4, a significant number of high-dose males minimally resisted handling with vocalizations (8/16 vs. 1/16 for controls); no other differences in behavior were observed during the FOB at any age. Results of motor activity, auditory startle response and learning and memory testing were not affected by treatment. Fixed brain weight at the terminal necropsy was significantly decreased in females at 200 ppm. Morphometric measurements were unaffected by treatment at the PND 21and terminal necropsies.

The maternal LOAEL is 200 ppm (16.1 mg/kg/day) based on decreased body weight, body weight gain, and food consumption. The maternal NOAEL is 80 ppm (6.78 mg/kg/day).

The offspring LOAEL is 200 ppm (16.1 mg/kg/day) based on decreased body weight and body weight gain during pre-weaning and post-weaning in males and females, increased incidence of vocalizations (males) during FOB handling, and decreased fixed brain weight in females at the terminal necropsy. The offspring NOAEL is 80 ppm (6.78 mg/kg/day).

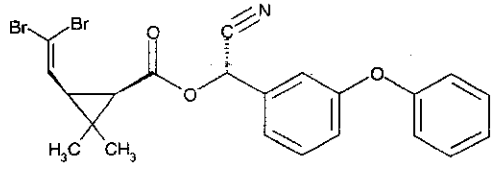
This study is classified as **Acceptable/Non-Guideline** and may be used for regulatory purposes. It does not, however, satisfy the guideline requirement for a developmental neurotoxicity study in rats (OPPTS 870.6300, §83-6) OECD 426 (draft)) due to the pending review of the positive control data.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. **Test material:** Deltamethrin

Description:	White solid
Batch #:	2350014
Purity:	98.8% a.i. (February 2004); 99.6% a.i. (February 2006)
Compound stability:	Stable at room temperature (~ 25 ± 5°C).
CAS # of TGAI:	52918-63-5
Structure:	

2. **Vehicle:** The test article was administered in the feed.

3. **Test animals (P):**

Species:	Rat
Strain:	Wistar HAN CRL:WI (GLX/BRL/HAN) IGSBR
Age at mating:	At least 14 (males) or 12 (females) of age at co-housing
Wt. after acclimation period:	Females: 186.4-223.4 g; males had no specific weight requirements
Source:	Charles River Laboratories, Inc., Raleigh, NC
Housing:	Individually in stainless steel cages, except with one male each during co-habituation with deodorized cage board in bedding tray; individually in plastic cages with corn cob bedding during gestation and lactation
Diet:	Purina Mills Rodent Lab Chow 5002 in meal form *ad libitum*, except during neurobehavioral testing
Water:	Tap water *ad libitum*
Environmental conditions:	Temperature: 18-26°C Humidity: 30-70% Air changes: 10/hour at minimum Photoperiod: 12 hrs dark/12 hrs light
Acclimation period:	Six days

B. PROCEDURES AND STUDY DESIGN:

1. **In life dates:** Start: September 19, 2004; End: February 4, 2005.

2. **Study schedule:** Maternal animals were mated with untreated males. The test substance was administered to the dams from gestation day (GD) 6 through lactation day (LD) 21. Pups were weaned on postnatal (PND) 21, after which time maternal animals were killed. F_1 pups remained on study until either PND 21 or 75 (± 5 days).

3. **Mating procedure:** One male and one female were co-housed for a maximum of five consecutive days. Each morning during the co-habituation phase, the dams and cages were examined for a vaginal plug and vaginal smears were taken to check for the presence of sperm. The day on which insemination was observed in the vaginal smear was designated day 0 of gestation (GD 0). On GD 0, the female was removed and housed individually in a plastic nesting cage.

4. **Animal assignment**: Mated females were assigned to the control or an exposure group in sequence, as they were determined to be inseminated. Offspring were randomly assigned to testing subgroups at the time of litter standardization on PND 4 (Table 1).

TABLE 1. Study design

Experimental parameter	Dietary concentration (ppm)			
	0	20	80	200
Maternal animals				
No. of maternal animals assigned	30	30	30	30
FOB (GD 13, 20/ LD 11, 21)	30/10	30/10	30/10	30/10
Offspring				
Detailed clinical observations/FOB (PND 4, 11, 21, 35(±1), 45(±1), 60(±2))	16 (min.10)/sex	16 (min.10)/sex	16 (min.10)/sex	16 (min.10)/sex
Motor activity (PND 13, 17, 21, 60(±2), 120(±5))	15-16 (min. 10)/sex	14-16 (min. 10)/sex	15-16 (min. 10)/sex	15-16 (min. 10)/sex
Auditory startle habituation (PND 22, 60(±2))	16 (min. 10)/sex	16 (min. 10)/sex	16 (min. 10)/sex	16 (min. 10)/sex
Learning and memory (PND 22/29, 60/67(±2))	15-16 (min. 10)/sex	16 (min. 10)/sex	15-16 (min. 10)/sex	16 (min. 10)/sex
Brain weight PND 21 PND 75(±5)	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex
Neuropathology PND 21 PND 75(±5)	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex	 10/sex 10/sex
Whole brain assay for deltamethrin	Max. 6 litters	N.A.	N.A.	Max 6 litters
Blood acetylcholinesterase	N.A.	N.A.	N.A.	N.A.
Brain acetylcholinesterase	N.A.	N.A.	N.A.	N.A.

N.A. = Not applicable (Brains were not analyzed for deltamethrin and cholinesterase activity was not measured.)

5. **Dose selection rationale:** The rationale for dose selection was based, in part, on the results of a two-generation reproduction study (MRID 44398101) in Sprague-Dawley rats at dietary levels of 0, 5, 20, 80 and 320 ppm. In the P_0 generation females, severe toxicity, including decreased body weight gain beginning on day 8, one death prior to mating and clinical signs during lactation, were observed at 320 ppm. Effects in the F_1 generation before weaning included reduced body weight at birth and throughout lactation, as well as pup deaths before weaning, at 320 ppm. No treatment-related findings were observed at the lower dietary levels.

   A pilot study was conducted to verify exposure of offspring during lactation [PND 10 to 16] and to determine how well Wistar rats would tolerate exposure to a dietary concentration of 250 ppm from GD 6 through lactation day (LD) 16. In that study, the 250 ppm concentration was reduced during lactation to compensate for increased feed consumption and thereby maintain a consistent mg/kg/day dosage throughout the exposure period. An increased incidence of pup loss, including cannibalization by the dam, during the first week of lactation at 250 ppm, indicated excessive toxicity to either the dam or offspring. Results from the brain tissue analyses conducted on PNDs 10, 14 and 16 demonstrated that the offspring were exposed to deltamethrin during lactation. Based on these results, the dietary levels selected for the present study were 0, 20, 80 and 200 ppm, with adjustments during lactation to maintain a consistent dosage throughout the period of exposure.

6. **Dosage administration:** All doses were administered in the diet to maternal animals on GD 6 through LD 21. For gestation, the nominal 20, 80 and 200 ppm dietary levels averaged 106%, 105% and 107% of the nominal concentrations, respectively. During lactation, dietary levels were adjusted to achieve a more consistent mg/kg/day dosage throughout the exposure period, since food consumption increases during that period.

7. **Dosage preparation and analysis:** Diet formulations were prepared weekly by mixing appropriate amounts of the test substance in the diet and were stored at freezer conditions. Dietary concentrations were not adjusted to correct for the percent active ingredient in the test substance but were adjusted during lactation, relative to gestation, to maintain a more constant level exposure (mg/kg/day).

Homogeneity (top, middle and bottom) was evaluated once prior to the study initiation at a range of concentrations that bracketed those used in the study (i.e., 3 and 1000 ppm). Stability of the test substance in the diet was evaluated for a period of 7 days at room temperature and 42 days at freezer conditions prior to the start of the study. During the study, samples of each batch of feed were analyzed to establish dietary concentrations.

Results:

Homogeneity analysis: The mean concentrations (relative standard deviations) of the 3 and 1000 ppm diet formulations were 101% (2.5%) and 102% (2.2%) of the nominal value, respectively.

Stability analysis: After 7 days at room temperature, the 3 and 1000 ppm concentrations were 100% and 103% of the initial concentration, respectively. After 42 days in the freezer, the concentration of the 3 and 1000 ppm formulations were 96% and 99%, respectively, if the initial concentration.

Concentration analysis: During gestation, the nominal 20, 80 and 200 ppm dietary levels averaged 106%, 105% and 107% of the nominal concentrations, respectively. Due to increased food consumption during lactation, dietary levels were adjusted to achieve a more consistent dosage (mg/kg/day) throughout the period of exposure. The nominal dietary levels and analytical results were as follows:

Lactation Week	1			2			3		
Nominal Concentration (ppm)	11	42	105	9	35	87	7	29	71
Mean Concentration (ppm)[1]	11.3	45.8	107	NA	NA	NA	6.57	27.7	65.8
% Nominal	103	109	102	NA	NA	NA	94	96	93

[1] Analytically confirmed.
NA=Not Applicable; the batch of feed at each of the three dietary levels was not analyzed at week 2 of lactation.

The analytical data indicated that the mixing procedure was adequate and that the difference between nominal and actual dosage to the study animals was acceptable.

C. OBSERVATIONS:

1. **In-life observations:**

a. **Maternal animals**: The animals were observed cage-side for clinical signs at least once daily. These observations characterized mortality, behavioral changes and overt toxicity. A physical examination was conducted once daily from the initiation of exposure (GD 6) through LD 21. The observations were performed by an individual who was aware of the animal's dosage group assignment.

All dams presumed to be pregnant (approximately 30/group) in each group were observed outside the home cage at least twice during gestation (days 13 and 20). A minimum of 10 dams/dietary level that were maintained on study with suitable litters were also observed on LDs 11 and 21. The Functional Observational Battery (FOB) examinations were performed under standard animal room conditions (temperature, relative humidity, etc.) and included observations in the home cage, during handling, and outside the home cage in an open field (one minute) using standardized procedures, as presented in the table below. Testing was performed by technicians who were unaware of each animal's dose assignment. The laboratory maintains evidence of inter-observer reliability for individuals who conduct the examinations.

	FUNCTIONAL OBSERVATIONS
X	Signs of autonomic function, including: 1) Ranking of degree of lacrimation and salivation, with range of severity scores from none to severe 2) Presence or absence of piloerection and exophthalamus, 3) Ranking or count of urination and defecation, including polyuria and diarrhea 4) Pupillary function such as constriction of the pupil in response to light, or a measure of pupil size 5) Degree of palpebral closure, e.g., ptosis.
X	Description, incidence, and severity of any convulsions, tremors, or abnormal movements.
X	Description and incidence of posture and gait abnormalities.
X	Description and incidence of any unusual or abnormal behaviors, excessive or repetitive actions (stereotypies), emaciation, dehydration, hypotonia or hypertonia, altered fur appearance, red or crusty deposits around the eyes, nose, or mouth, and any other observations that may facilitate interpretation of the data.

Individual maternal body weight and food consumption were measured once weekly during gestation and lactation, as follows: GDs 6-13, 13-20 and LDs 0-7, 7-14 and 14-21. Fresh feed and clean feeders were provided weekly. The average daily intake of the active ingredient, expressed as mg a.i. consumed/kg body weight, was calculated using the following relationship: [µg of a.i./g feed/1000] X [feed consumed (g/kg bw/day)].

b. **Offspring**:

1. **Litter observations**: Each dam was evaluated daily for evidence of delivery from GD 20 to the completion of delivery, which was designated LD 0 for the dam and PND 0 for the offspring. The number of pups delivered and the pup status at birth were recorded for each litter. If a dam delivered fewer than three pups per sex or if the litter size decreased to less than seven pups by PND 4, the dam and litter were sacrificed without necropsy (except for litters reserved to assay brain for deltamethrin). Live pups were counted, sexed and weighed individually on PNDs 0, 4, 11, 17 and 21. Daily throughout lactation, offspring were examined cage-side for gross signs of mortality, morbidity and clinical

signs of toxicity. Detailed observations for clinical signs were made once daily before weaning and once weekly thereafter.

On PND 4, litters were standardized to a maximum of 8 pups/litter (4/sex/litter, as nearly as possible) using a random selection technique. If there were more than 23 acceptable litters for any group, the surplus litters were sacrificed on PND 4 after weighing and without necropsy. Culled dams and pups were sacrificed by CO_2 asphyxiation and decapitation, respectively. Dams with insufficient litters were also sacrificed by CO_2 asphyxiation.

2. **Developmental landmarks**: Beginning on postnatal day 38, male offspring were examined daily for preputial separation. Beginning on postnatal day 29, female offspring were examined daily for vaginal patency. The age of onset and body weight at that time were recorded. All pups were also tested for pupil constriction on PND 21.

3. **Postweaning observations:** After weaning on postnatal day 21, pups were weighed once weekly, as well as when vaginal patency and balanopreputial separation was first evident, with detailed observations for clinical signs performed once weekly. Food consumption was not measured after weaning.

4. **Neurobehavioral evaluations**: Observations and the schedule for those observations are summarized as follows from the report. The test room used for motor activity, auditory startle habituation and passive avoidance conditioning was a standard animal room that was set to be maintained on the same light:dark cycle as the room in which the animals were housed, with tests conducted during the light phase, while water maze testing was performed in the room where the animals were housed. The order of testing and assignment of animals to specific test devices was semi-random, such that groups were balanced across test times and devices and no animal was tested more than once in the same device. An exception was that animals were tested in the same water maze on both occasions, as per standard procedure.

   i. **Functional observational battery (FOB):** On PNDs 4, 11, 21, 35 (±1 day), 45 (±1 day), 60 (±2 days), approximately 16 offspring/sex/group (minimum of one male or one female from each litter) were examined outside the home cage in an FOB assessment, as appropriate for the developmental stage being observed. The same parameters assessed in the maternal FOB were examined for offspring, except that neonates (i.e., PNDs 4 and 11) were not evaluated in the open field.

   ii. **Motor activity testing**: Motor activity was evaluated in approximately 16 rats/sex/dose (minimum of ten pups/sex/dietary dose) on PNDs 13, 17, 21, 60±2 and 120±5. Animals were tested at 120 days of age to address possible questions raised by published findings in mice that were tested at this age, following exposure to deltamethrin during lactation.[1] Activity was monitored for 60 minutes (six ten-

[1] Eriksson, P., A. Fredriksson, 1991. Neurotoxic effects of two different pyrethroids, bioallethrin and deltamethrin, on immature and adult mice: Changes in behavioral and muscarinic receptor variables. Toxicol Appl Pharmacol 108:78-85.

minute intervals) in figure-eight mazes. Each maze consisted of eight infrared emitter/detector pairs (three in each of the figure eight alleys and one in each of the blind alleys). A Columbus Instruments Universal Maze Monitoring System was used for data collection. Broad-spectrum background noise was provided throughout the test to minimize acoustical variation during testing. The uniformity of light intensity over each maze was verified each day. Motor activity was measured as the number of beam interruptions that occurred during each session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was evaluated as a decrement in activity over consecutive intervals of each session.

iii. **Auditory startle reflex habituation:** Auditory startle reflex habituation testing was performed on 16 rats/sex/dose (minimum of 10 offspring/sex/dose) on PNDs 22 and 60 (±2 days) using an automated system. Groups of four animals were tested simultaneously within the startle system enclosure. The enclosure was ventilated, lined with sound-attenuating and vibration-absorbing material and housed a speaker mounted in the ceiling to provide the eliciting stimulus (a 50-msec burst of white noise at approximately 118 dB). The enclosure housed four load cell/force transducer assemblies that measured the startle response. The test session consisted of 50 trials that began following a 5-minute adaptation period at ambient noise levels. The rats were then presented with the startle-eliciting stimulus at 10-sec intervals. Data collection began with the presentation of the stimulus and continued for 200 msec. The analog signal for each response output (measured in mV) was digitized at one kHz (one sample/msec for 200 msec) and converted to grams using a calibration curve for each load cell. Peak response amplitude (g) and latency (msec) measurements were taken from each animal's response curve. Baseline was defined as the average force (g) exerted on the platform during the first eight msec following the onset of the stimulus. This baseline was taken to represent an approximate body weight measurement to verify that the equipment was functioning properly. Response amplitude was defined as the maximum value of the average curve minus the baseline. Latency to peak was the time (msec) following the onset of the stimulus when the peak response occurred.

iv. **Learning and memory testing:** Learning and memory testing was performed in approximately 16 rats/sex/dose (minimum 10 offspring/sex/dose). The same set of animals was tested for passive avoidance (on PNDs 22 and 29) and water maze (PND 60±2 and again seven days later).

Postweaning – Passive avoidance: Animals were tested for acquisition on PND 22 and for retention on PND 29. Testing was conducted using an integrated system of equipment and computer programs from Coulbourn Instruments. Testing occurred in individual isolation cubicles, each with a single shuttle cage. Each shuttle cage (approximately 14 x 7 inches) was separated into two compartments of equal size by a wall that supported a centrally-located sliding door. The walls of one compartment were lined with black film (dark side) and the walls of the other compartment were illuminated with a high-intensity lamp. After adaptation, the animal was placed into the lighted compartment facing toward the light. After approximately 60 seconds, the light was illuminated and the door between the compartments was opened. When the

rat moved into the dark compartment, the door closed, a shock was delivered and the light was switched off. The rat was then returned to its cage until the next trial. If the rat did not cross to the dark compartment within 180 seconds, it was returned to its cage and given a latency score of 180. The procedure was repeated until either the rat remained in the lighted compartment for 180 seconds on two consecutive trials or until 15 trials elapsed, whichever occurred first. Animals that failed to reach criterion performance with 15 trials or failed to cross during the first two trials during acquisition were excluded from the retention phase. The test was repeated one week later. In the second trial, rats were placed in the illuminated compartment, given a 20-second acclimation period and then the latency to enter the dark side was recorded. Animals that either failed to reach criterion performance within 15 trials or failed to cross during the first trials during acquisition were excluded from the retention phase of the experiment. The dependent measures were the number of trials-to-criterion, latency to cross on Trial 1 and Trial 2 (learning phase only) and the number of rats/group that failed to reach criterion within 15 trials (learning phase only).

Adult (PND 60) Offspring – Water maze: The animals assigned to passive avoidance testing were also assigned to the water maze testing on PND 60±2 and again seven days later. Only animals that demonstrated acquisition within the 15-trial limit were tested for retention seven days later. The M-maze was constructed of Plexiglas with five inch wide corridors and contained approximately 7.5 inches of water maintained at 22±1EC. For each trial, the rat was placed in the starting position at the base of the M-maze stem, located between the two lateral arms. For the first trial (learning trial), the rat was required to enter both arms of the maze before being provided access to the exit ramp to escape the maze. The initial arm for the learning trial was the incorrect goal for the subsequent 15 trials (maximum). Rats that failed to make a correct goal choice within 60 seconds in any trial were guided to the correct goal with the exit ramp and removed from the maze. Between trials, the animals were kept in a transport cage for approximately 15±5 seconds. Each rat was required to reach a criterion of five consecutive errorless trials to terminate the test session. The maximum number of trials in a test session was fifteen. Latency (time in seconds to choose the correct goal or the maximum of 60 seconds) and the number of errors (incorrect turns in the maze) were recorded.

Dosage groups were compared for the following dependent measures: measures for acquisition included the number of trials-to-criterion, the average number of incorrect turns in maze for each trial; and latency to reach the correct goal on trial 2 (measure of short-term retention). Measures for retention included the number of trials to criterion, the average number of errors for each trial; and the latency to reach the correct goal on trial 1 (measure of long-term retention).

5. **Ophthalmology**: At approximately 50-60 days of age, ophthalmic examinations were conducted on a minimum of 10 rats/sex/group representing at least 20 litters selected for perfusion at study termination. Pupillary reflex was tested using a penlight or transilluminator after dilation of the pupils with a mydriatic. The conjunctiva, cornea and lens were examined with a slit lamp microscope either before or after pupillary

dilation. After dilation, the vitreous humor, retina, choroid and optic disc were examined using an indirect ophthalmoscope equipped with a condensing lens.

2. **Postmortem observations:**

a. **Maternal animals:** Dams were sacrificed by CO_2 asphyxiation on LD 21 after the weaning of their litters; necropsy was not conducted. Mated females that did not deliver a litter were sacrificed on GD 24 without necropsy.

b. **Offspring**: The offspring selected for brain weight or neuropathological evaluation were sacrificed on PND 21 or 75 (±5 days). All moribund pups were sacrificed and subjected to gross necropsy. Tissues were collected at the discretion of the study director. Randomly-selected animals that were used to measure fresh brain weight underwent a necropsy examination. Where required, the necropsy involved an examination of all organs, body cavities, cut surfaces, external orifices and surfaces, with all gross abnormalities recorded. Gross lesions in neural tissues or skeletal muscle were appropriately sampled for microscopic examination. Animals found dead underwent necropsy and were disposed of without collection of tissues. Pups selected for culling were sacrificed by decapitation and discarded without necropsy.

Animals selected for perfusion on PND 21and at study termination were anesthesized with an intraperitoneal dose of pentobarbital and then perfused via the left ventricle with a sodium nitrite flush (in phosphate buffer) followed by *in situ* fixation using universal fixative [1% (w/v) glutaraldehyde and 4% (w/v) EM-grade formaldehyde] in phosphate buffer. On PND 21, only the brain (with olfactory bulbs) was collected. At study termination, the brain and spinal cord, both eyes (with optic nerves), selected bilateral peripheral nerves (sciatic, tibial and sural), the gasserian ganglion, gastrocnemius muscle, both forelimbs and physical identifier were collected. All tissues were placed in 10% buffered formalin. The brain was weighed upon removal from the skull before placement in the formalin; the brain: body weight ratio was calculated.

Prior to sectioning for histology, the following brain measurements were made using a Vernier caliper: 1) anterior-to-posterior length of the cerebrum, extending from the anterior pole to posterior pole, exclusive of the olfactory bulbs; and 2) anterior-to-posterior length of the cerebellum, extending from the anterior edge of the cortex to the posterior pole. These measurements were performed by a technician who was aware of the dose assignments.

After the gross measurements, the brain was divided into eight coronal sections for microscopic examination. The eight sections were processed for paraffin embedding, sectioned and examined after staining with hematoxylin and eosin (H&E). Brain sections reserved for morphometric measurements (levels 3-5 and 7) were stained with luxol fast blue/cresyl violet. The following tissues were also collected from the perfused animals at terminal necropsy for embedding in paraffin and staining with H&E: three levels of the spinal cord (cervical, thoracic and lumbar), the cauda equina, eyes, optic nerves and gastrocnemius muscle. Dorsal root ganglia (including dorsal and ventral root fibers) from the cervical and lumbar swellings and gasserian ganglia were embedded in glycol

methacrylate (GMA), sectioned and stained with a modified Lee's stain. Peripheral nerve tissues (sciatic, tibial and sural nerves) were embedded in GMA resin and sectioned longitudinally. The sciatic nerve was also cut in cross section.

Only tissues from control and high-dose animals were examined for micropathology and morphometry. If no lesions were found, the other dose groups were not examined. Sections from all dose groups were coded and examined in randomized order without knowledge of the code. The frequency and severity of each lesion were determined before the code was broken and the data were evaluated for a dose-effect relationship.

Seven linear measurements of selected brain regions were taken, including the two gross measurements on the intact brain discussed above. The other five taken from the histologic sections using software calibrated with an ocular micrometer were as follows:

1. Frontal cortex thickness (forebrain) - measurement of the dorsal portion of the cerebral cortex within a coronal section passing through the region of the optic chiasm.

2. Parietal cortex thickness (forebrain) - measurement of the dorsolateral portion of the cerebral cortex within the coronal section taken through the optic chiasm.

3. Caudate putamen horizontal width (forebrain; maximum cross-sectional width) - measurement on the coronal section at the level of the optic chiasm.

4. Hippocampal gyrus thickness (midbrain) - mean of two measurements of the full width on both sides of the hippocampal gyrus from the ventral tail of the dentate gyrus to the overlying subcortical white matter.

5. Cerebellum height (cerebellum/pons) - measurement extending from the roof of the fourth ventricle to the dorsal surface.

D. DATA ANALYSIS:

1. **Statistical analyses:** All statistical tests used a significance level of $p \leq 0.05$, except for Bartlett's, which used $p \leq 0.001$. Continuous data were assessed for equality of variance using Bartlett's test. Group means with equal variances were analyzed using an Analysis of Variance (ANOVA), followed by a Dunnett's test if a significant F-value was determined in the ANOVA. If there were unequal variances, the data were analyzed using nonparametric statistical procedures (Kruskal-Wallis ANOVA followed by the Mann-Whitney U test for between-group comparisons).

   FOB continuous data were analyzed using an ANOVA, with *post-hoc* comparisons using Dunnett's test. Categorical data were analyzed using General Linear Modeling and Categorical Modeling Procedures, with *post-hoc* comparisons using Dunnett's test and an Analysis of Contrasts, respectively.

   Motor and locomotor activity (total session activity and activity for each 10-minute interval) were analyzed using ANOVA procedures. Session activity data for the four test

occasions were analyzed using an ANOVA to determine if there was a significant day-by-treatment interaction. If so, Dunnett's test was used to determine if the treated group was significantly different from the control. Interval data were subjected to a Repeated-Measures ANOVA, using both test interval and test occasion as repeated measures, followed by an ANOVA to determine if there was a significant treatment-by-interval interaction on each test occasion. If so, the data were analyzed using Dunnett's test to determine whether the treated group was significantly different from the control.

Auditory startle response amplitude data (peak amplitude) for the three occasions were first analyzed using an ANOVA. If there was a significant group effect, Dunnett's test was used to determine if the treated group was significantly different from the control. The response amplitude data for each block of ten trials (five blocks/test session) were analyzed using a Repeated-Measures ANOVA, using test block as the repeated measure. If there was a significant group-by-block interaction, the block values were analyzed using Dunnett's test to determine if the treated group was significantly different from the control.

Passive avoidance latency data were analyzed using a Wilcoxon Test for time to failure. The number of trials to criterion was analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats not meeting the criterion level in the learning phase was analyzed as incidence data.

Water maze latency data were analyzed by a univariate ANOVA, with *post-hoc* analysis using Dunnett's test. The number of trials to criterion and the number of errors were analyzed using Kruskal-Wallis and Wilcoxon tests for the acquisition phase and Fisher's Exact Test for retention. The number of rats not meeting the criterion level in the learning phase was analyzed as incidence data.

Pathology data were screened for potential effects and then evaluated using the table below.

Data Type	Data	Statistical Tests
	Organ Weight	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)
	Gross Brain Measurements	Bartlett's for Homogeneity, with ANOVA or Kruskal-Wallis (1)
	Microscopic Brain Measurements	ANOVA and/or T-test (2)
Frequency	Ophthalmology	Visually Screened (3)
	Gross Pathology	Visually Screened (3)
	Micropathology	Chi-Square Fisher's Exact Test

(1) ANOVA was used if data were homogeneous; Kruskal-Wallis was used if data were nonhomogeneous.
(2) A T-test, 2-tailed, was used for two-group comparisons; an ANOVA was used for multiple-group comparisons.
(3) If potential compound effects were suspected, then a Chi-square and one-tailed Fisher's Exact test were used.
All statistical tests used a significance level of $p \leq 0.05$, except for Bartlett's test, which used $p \leq 0.01$.

2. Indices:

a. **Reproductive indices:** The following reproductive indices were calculated from breeding and parturition records of animals in the study:

$$\text{Mating index (\%)} = \frac{\text{number of inseminated females}}{\text{number of females cohoused with males}} \times 100$$

$$\text{Female fertility index (\%)} = \frac{\text{number of pregnant females}}{\text{number of inseminated females}} \times 100$$

b. **Offspring viability indices:** The following viability (survival) indices were calculated from lactation records of litters in the study:

$$\text{Live birth index (\%)} = \frac{\text{number of live born pups at birth per litter}}{\text{total number of pups born per litter}} \times 100$$

$$\text{Viability index (\%)} = \frac{\text{number of live pups on day 4 preculling per litter}}{\text{number of live pups born per litter}} \times 100$$

$$\text{Lactation index (\%)} = \frac{\text{number of live pups on day 21 per litter}}{\text{number of live pups on day 4 post-culling per litter}} \times 100$$

3. **Positive and historical control data:** References were made to positive control studies for neurobehavioral testing (motor activity, acoustic startle habituation, passive avoidance and water maze) but no actual data were presented. The only historical control data submitted with the study were brain morphometric measurements, but the bases for the stated values were not provided. It is assumed that complete data sets have been submitted to EPA and are considered acceptable.

II. RESULTS:

A. PARENTAL ANIMALS:

1. **Mortality and clinical and functional observations:** No deaths were reported in dams during gestation and lactation. No treatment-related clinical signs were observed. Incidental findings during gestation included scab formation in three high-dose females and alopecia in two low- and one high-dose females. Incidental findings observed during lactation included scab formation in two high-dose females and alopecia in one or two females, each from various dose groups, with no relationship to dietary level.

   During the FOB, no treatment-related findings were observed.

2. **Body weight and food consumption:** Selected group mean body weight, body weight gain and food consumption values for pregnant or nursing dams are summarized in Table 2. During gestation, body weight was significantly decreased (6-7%) in females at 200 ppm on GDs 13 and 20. Body weight gain during GDs 0-20 and food consumption during GDs 6-13

were significantly decreased (both 17%) for high-dose females. During lactation, body weight (6-8%) and food consumption (9%) were decreased in high-dose females on LD 0-7 but were comparable to the control group for the remainder of lactation. Body weight gain during lactation (LDs 0-21) was increased (dose response) in treated females.

TABLE 2. Mean (±SD) maternal body weight, body weight gain and food consumption [a]

Observations/study week	Dietary concentration (ppm)			
	0	20	80	200
Gestation				
Mean body weight (g) Gestation day 0	213.5 ± 1.8	210.9 ± 1.7	208.1 ± 2.8	209.2 ± 1.9
Mean body weight (g) Gestation day 6	237.3 ± 2.2	234.3 ± 2.0	231.3 ± 3.0	232.5 ± 2.3
Mean body weight (g) Gestation day 13	263.7 ± 2.6	260.1 ± 2.4	255.3 ± 3.4	247.1** ± 2.6 (↓6)[b]
Mean body weight (g) Gestation day 20	326.1 ± 3.7	320.2 ± 3.2	316.8 ± 5.0	302.7** ± 3.2 (↓7)
Mean weight gain (g) Gestation days 0-20	112.6 ± 2.2	109.3 ± 2.2	108.7 ± 2.9	93.5* ± 2.2 (↓17)
Mean food consumption (g/animal/day) Gestation days 6-13	20.7 ± 0.5	19.9 ± 0.3	19.6 ± 0.4	17.2 ** ± 0.5 (↓17)
Mean food consumption (g/animal/day) Gestation days 13-20	22.0 ± 0.4	21.9 ± 0.4	23.0 ± 0.8	21.6 ± 0.5
Lactation				
Mean body weight (g) Lactation day 0	252.3 ± 3.5	248.9 ± 2.5	244.1 ± 4.1	233.5** ± 2.5 (↓7)
Mean body weight (g) Lactation day 4	270.3 ± 2.9	264.0 ± 3.2	258.3 ± 4.2	248.5** ± 3.1 (↓8)
Mean body weight (g) Lactation day 7	276.3 ± 3.0	275.2 ± 3.7	269.0 ± 5.1	258.8** ± 3.1 (↓6)
Mean body weight (g) Lactation day 21	284.8 ± 3.0	285.9 ± 3.2	285.1 ± 4.2	278.6 ± 3.3
Mean weight gain (g)[c] Lactation days 0-21	32.5	37.0	41.0	45.1
Mean food consumption (g/animal/day) Lactation days 0-7	36.3 ± 1.3	34.7 ± 1.3	35.1 ± 2.0	33.2 ± 1.4 (↓9)
Mean food consumption (g/animal/day) Lactation days 7-14	51.1 ± 1.1	48.7 ± 0.9	48.8 ± 1.3	49.2 ± 1.4
Mean food consumption (g/animal/day) Lactation days 14-21	65.4 ± 2.1	62.0 ± 3.4	62.8 ± 1.4	61.3 ± 1.2

[a] Data obtained from Text Table 4, page 39, MRID 46814301.
[b] Number in parentheses is percent difference from control; calculated by reviewer.
c Calculated by the reviewer without standard deviation or statistical analysis.
* Statistically different from control, $p<0.05$.
** Statistically different from control, $p<0.01$.
N = 27-30 during gestation; 22-30 during lactation

3. **Test substance intake:** The average consumption of test material for females that received the diet formulations during gestation, with adjustments during lactation, are presented in Table 3. Based on these results, the average daily intake of active ingredient during gestation and lactation was 0, 1.64, 6.78 and 16.1 mg/kg/day.

TABLE 3. Mean maternal test substance intake (mg/kg body weight/day) [a]			
Period	Dietary concentration (ppm)		
	20	80	200
Gestation			
Gestation days 6-13	1.8 ± 0.02	7.1 ± 0.12	15.7 ± 0.37
Gestation days 13-20	1.8 ± 0.02	7.5 ± 0.22	18.6 ± 0.40
Lactation			
Lactation days 0-7	1.6 ± 0.07	6.6 ± 0.41	15.2 ± 0.57
Lactation days 7-14[b]	1.6 ± 0.04	6.6 ± 0.13	16.6 ± 0.47
Lactation days 14-21	1.4 ± 0.08	6.1 ± 0.12	14.4 ± 0.25

[a] Data obtained from Text Table 5, page 40, MRID 46814301.
[b] Value estimated, based on the average percent nominal concentration measured for all other weeks.
N = 22-30.

4. **Reproductive performance:** Of the 30 females/group, two in the control and one in the 20 ppm group were not pregnant. The fertility index was 93.3%, 96.7%, 100.0% and 100.0% in the control, low-dose, mid-dose and high-dose groups, respectively. The mean duration of gestation was 21.8, 21.8, 21.7 and 21.7 days for the respective groups. Results for the maternal animals are summarized from the report in Table 4

TABLE 4. Reproductive performance [a]				
Observation	Dietary concentration (ppm)			
	0	20	80	200
Number mated	30	30	30	30
Maternal wastage				
No. of dams not pregnant	2	1	0	0
No. of dams delivering dead pups	0	1	1	0
No. of dams with pre-mature delivery	0	0	0	0
Mating index	100.0	100.0	100.0	100.0
Fertility index (%)	93.3	96.7	100.0	100.0
Mean (±SE) gestation duration (days)[b]	21.8 ± 0.10 [22.0] (21.0-23.0)	21.8 ± 0.11 [22.0] (21.0-23.0)	21.7 ± 0.12 [22.0] (21.0-23.0)	21.7 ± 0.12 [22.0] (21.0-23.0)

[a] Data obtained from Text Table 6, page 41, MRID 46814301.
[b] Values are mean ± standard error, [median] and (range).

5. **Maternal postmortem results**: Necropsies were not conducted on parental animals.

B. OFFSPRING:

1. **Viability and clinical signs**: Litter size and viability (survival) results from pups during lactation are summarized from the report in Table 5. Litter parameters and pup viability were not affected by treatment. The sex ratio (% males) on PND 0 (calculated by the reviewer) was decreased at 200 ppm (44.6% vs. 48.8% in controls). No treatment-related clinical signs were observed in offspring during lactation. Bruising on the face, back and/or body were reported in all groups.

No treatment-related clinical signs were observed after weaning when exposure was discontinued. Incidental findings included urine stain (one control female; one mid- and two high-dose males), perianal stain (one high-dose male), red nasal stain (one control and mid-dose male, each), general ocular opacity (one mid-dose male), exophthalmus (one high-dose male, unilateral) and dehydration (one control female). Dermal lesions reported in control and treated animals were also considered incidental. One low-dose male had a firm dermal palpable mass on the right side of the abdomen on PND 43 and was found dead on PND 56. One low-dose male was found dead on PND 56, one control female was found dead on PND 86, and one low-dose female was found dead on PND 66; a cause of death was not given for any animal.

TABLE 5. Litter size and viability [a]

Observation	Dietary concentration (ppm)			
	0	20	80	200
Number of litters	23	23	23	23
Total number born	265	249	259	258
Total number pups missing	1	6	2	0
Litters with pups missing	1	2	2	0
Total number pups found dead	0	1	1	1
Litters with pups found dead	0	1	1	1
Total number pups cannibalized	0	0	0	0
Litters with pups cannibalized	0	0	0	0
Litter size Mean ± S.E.	11.5 ± 0.27	10.8 ± 0.38	11.3 ± 0.30	11.2 ± 0.41
Stillborn pups Number Percentage	 0 0.0	 3 1.2	 0 0.0	 0 0.0
Sex ratio (% males) – PND 0 [b]	48.8	50.2	50.5	44.6
Mean number viable pups				
Birth	12	11	11	11
Day 4 [c]	12	10	11	11
Day 4 [d]	8	8	8	8
Day 21	8	8	8	8
Live birth index % ± S.E.	100.0 ± 0.00	98.9 ± 1.09	100.0 ± 0.00	100.0 ± 0.00
Viability index % ± S.E.	100.0 ± 0.00	97.9 ± 1.83	98.8 ± 0.64	99.7 ± 0.29
Lactation index % ± S.E.	99.5 ± 0.54	99.5 ± 0.54	100.0 ± 0.00	100.0 ± 0.00

[a] Data obtained from Text Table 7, page 42, MRID 46814301.

[b] Calculated by the reviewer based on data from pages 235-238.

[c] Before standardization (culling).

[d] After standardization (culling).

2. **Body weight**: Body weight was significantly decreased in males (7-10%) and females (6-9%) at 200 ppm beginning on PND 4. The lower body weight was a result of decreased body weight gain during PNDs 1-4 and 4-11. For high-dose males, body weight gain during PNDs 1-4 and 4-11 was reduced by 15% and 10%, respectively, compared with the controls resulting in overall weight gain during lactation reduced by 8%. For high-dose females, body weight gain during PNDs 1-4 and 4-11 was reduced by 18% and 10%, respectively,

compared with the controls resulting in overall weight gain during lactation reduced by 7%. Selected mean preweaning pup body weight data are presented in Table 6.

TABLE 6. Mean (±SD) pre-weaning pup body weight and body weight gain[a]

Postnatal day	Dietary concentration (ppm)							
	0	20	80	200	0	20	80	200
	Males				Females			
Body Weight (g)								
1	6.0 ± 0.1	6.1 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.8 ± 0.1	5.6 ± 0.1	5.5 ± 0.1
4 [b]	9.9 ± 0.2	10.4 ± 0.2	9.6 ± 0.3	9.1 ± 0.3 (↓8)	9.6 ± 0.2	10.0 ± 0.2	9.1 ± 0.3	8.7* ± 0.3 (↓9)
4[c]	10.0 ± 0.2	10.4 ± 0.2	9.6 ± 0.3	9.1* ± 0.3 (↓9)	9.6 ± 0.2	10.0 ± 0.3	9.2 ± 0.3	8.8 ± 0.3 (↓8)
11	24.8 ± 0.5	25.4 ± 0.5	24.2 ± 0.5	22.4** ± 0.6 (↓10)	24.2 ± 0.5	24.6 ± 0.6	23.3 ± 0.6	22.0* ± 0.6 (↓9)
17	38.0 ± 0.6	38.4 ± 0.7	37.2 ± 0.8	35.4* ± 0.7 (↓7)	36.7 ± 0.5	37.1 ± 0.7	35.6 ± 0.8	34.6* ± 0.7 (↓6)
21	49.2 ± 0.9	49.1 ± 0.8	47.9 ± 1.0	45.5* ± 0.9 (↓8)	47.9 ± 0.7	47.4 ± 0.9	45.7 ± 1.1	44.6* ± 0.9 (↓7)
Body Weight Gain (g)[d]								
1-4 [b]	3.9	4.3	3.7	3.3 (↓15)	3.9	4.2	3.5	3.2 (↓18)
4[c]-11	14.8	15.0	14.6	13.3 (↓10)	14.6	14.6	14.1	13.2 (↓10)
11-17	13.2	13.0	13.0	13.0	12.5	12.5	12.3	12.6
17-21	11.2	10.7	10.7	10.1	11.2	10.3	10.1	10.0
1-21	43.2	43.0	42.0	39.7 (↓8)	42.2	41.6	40.1	39.1 (↓7)

[a] Data obtained from Text Table 8, page 44, MRID 461814301.

[b] Before standardization.

[c] After standardization

[d] Calculated by the reviewer without standard deviation and statistical analysis.

Significantly different from control value: *p<0.05; **p<0.01.

N = 23

During postweaning, the decreased body weight in the high-dose animals that developed during lactation persisted for one week in females (7%) and for four weeks in males (4-8%). Selected mean postweaning offspring body weight data are presented in Table 7.

TABLE 7. Mean (±SD) post-weaning pup body weights (g) [a]

PND	Dietary concentration (ppm)							
	0	20	80	200	0	20	80	200
	Males				Females			
28	79.6±7.9	81.3±6.1	78.0±7.0	73.4*±7.2 (↓8)	78.3±6.8	79.1±5.4	75.0±6.3	73.1*±5.4 (↓7)
35	125.5±9.5	127.3±7.9	122.2±9.1	118.6*±8.7 (↓5)	113.0±7.1	115.6±5.5	110.6±7.1	109.5±5.1
42	173.3±11.9	173.7±8.9	168.5±10.4	164.3*±10.1 (↓5)	136.2±8.2	138.4±5.6	134.1±6.7	132.7±5.9
49	216.9±14.3	217.9±10.7	211.5±12.5	207.5*±11.0 (↓4)	150.6±9.3	155.1±6.3	152.8±7.5	151.5±7.0
70	321.2±23.1	324.5±16.6	314.6±20.1	311.7±15.5	191.1±11.3	195.1±8.2	190.4±11.4	191.3±7.7

[a] Data obtained from Text Table 9, page 45, MRID 46814301.
* Significantly different from control value, $p<0.05$.
PND = postnatal day.
N = 23

3. Developmental landmarks:

a. Sexual maturation: The data are presented in Table 8. The onset of balanopreputial separation was significantly delayed in high-dose males as compared to controls. The average age of onset was 43.5, 44.0, 44.3 and 45.1 days for the 0, 20, 80 and 200 ppm dietary groups, respectively. However this finding was attributed to the lower body weight of these animals compared with the controls (Table 8) The onset of vaginal patency was not affected by treatment. The average age of onset was 32.0, 31.9, 32.8 and 33.4 for the 0, 20, 80 and 200 ppm groups, respectively. The difference from control at the 200 ppm dietary level was not attributed to treatment since there was no statistical difference. Additionally, the average age of onset for the controls was below the range of historical control while the high-dose females were within the historical control range (32.6 to 34.6 days).

TABLE 8. Mean (±SD) age and body weight at sexual maturation [a]

Parameter	Dietary concentration (ppm)			
	0	20	80	200
N (M/F)	23/23	23/23	23/23	23/23
Males				
Preputial separation (days)	43.5 ± 0.31	44.0 ± 0.39	44.3 ± 0.29	45.1* ± 0.48
% of pups reaching criterion	100	100	100	100
Mean (±SD) post-weaning pup body weights (Day 35)	125.5±9.5	127.3±7.9	122.2±9.1	118.6*±8.7 (↓5)
Mean (±SD) post-weaning pup body weights (Day 42)	173.3±11.9	173.7±8.9	168.5±10.4	164.3*±10.1 (↓5)
Females				
Vaginal opening (days)	32.0 ± 0.32	31.9 ± 0.32	32.8 ± 0.36	33.4 ± 0.50
% of pups reaching criterion	100	100	100	100

[a] Data obtained from Text Table 10, page 46, MRID 46814301.
** Significantly different from control value, $p<0.01$.

b. **Other landmarks**: Pupil constriction in response to penlight was apparent in all groups on PND 21. Body weight was supposed to be measured on the day criterion was met but no data were reported.

4. **Behavioral assessments:**

a. **Functional observational battery:** The only possible treatment-related effect was evident in high-dose males on PND 4 when a statistically significant number of animals minimally resisted handling with vocalizations (8/16 vs. 1/16 in control). Incidental findings included red nasal stain in one control male (PNDs 35 and 45) and scabs in one low- and high-dose male, each and two mid-dose males, all occurring on PND 60. The data are presented in Table 9.

TABLE 9. FOB observations [number (percentage)] in male F_1 animals [a]

Observation	Dietary concentration (ppm)			
	0	20	80	200
PND 4				
N	16	16	16	16
Ease of removal				
Minimal Resistance	15 (94%)	15(94%)	14 (88%)	8* (50%)
Minimal Resistance with vocalizations	1 (6%)	1 (6%)	2 (13%)	8* (50%)
PND 35				
N	16	16	16	16
Handling – Stains - Nasal				
Not observed for any color	15 (94%)	16 (100%)	16 (100%)	16 (100%)
Red	1 (6%)	0 (0%)	0 (0%)	0 (0%)
PND 45				
N	16	16	16	16
Handling – Stains - Nasal				
Not observed for any color	15 (94%)	16 (100%)	16 (100%)	16 (100%)
Red	1 (6%)	0 (0%)	0 (0%)	0 (0%)
PND 60				
N	16	16	16	16
Handling – Scabs				
Not observed	16 (100%)	15 (94%)	14 (88%)	15 (94%)
Present	0 (0%)	1 (6%)	2 (13%)	1 (6%)

[a] Data obtained from Text Table 11, page 47 and page 132, MRID 46814301.
* Statistically different ($p < 0.05$) from the control.
N = Number of animals

c. **Motor activity:** Summary motor and locomotor activity data are presented in Table 10. No treatment-related effects were observed. Gender-related differences were noted on PNDs 60 and 120 when higher levels of motor and locomotor activity were evident in females.

Interval motor and locomotor activity are presented in Tables 10a and 10b. Habituation was evident in both sexes at all ages, except PND 13, when activity levels were relatively low.

TABLE 10. Mean (±S.D.) motor and locomotor activity data (total activity counts for session) in F_1 rats [a]

Test Day	Dietary concentration (ppm)			
	0	20	80	200
Males				
Motor Activity				
PND 13	71 ± 56	92 ± 112	101 ± 79	65 ± 67
PND 17	180 ± 91	185 ± 132	222 ± 98	200 ± 106
PND 21	301 ± 136	261 ± 112	289 ± 58	268 ± 141
PND 60	511 ± 102	557 ± 106	538 ± 119	543 ± 109
PND 120	383 ± 66	401 ± 115	415 ± 142	398 ± 98
Locomotor Activity				
PND 13	8 ± 9	15 ± 22	10 ± 10	10 ± 13
PND 17	45 ± 27	47 ± 35	55 ± 28	50 ± 32
PND 21	93 ± 36	73 ± 27	92 ± 28	75 ± 38
PND 60	344 ± 71	384 ± 101	381 ± 102	383 ± 89
PND 120	255 ± 56	257 ± 72	281 ± 99	270 ± 61
Females				
Motor Activity				
PND 13	42 ± 34	40 ± 33	81 ± 65	61 ± 47
PND 17	213 ± 148	167 ± 99	192 ± 126	170 ± 114
PND 21	312 ± 108	300 ± 110	263 ± 78	260 ± 106
PND 60	696 ± 108	695 ± 253	710 ± 169	730 ± 185
PND 120	542 ± 131	546 ± 193	507 ± 167	580 ± 229
Locomotor Activity				
PND 13	7 ± 10	4 ± 6	9 ± 14	7 ± 10
PND 17	57 ± 49	50 ± 38	50 ± 34	46 ± 36
PND 21	91 ± 38	84 ± 31	79 ± 33	80 ± 36
PND 60	471 ± 112	438 ± 164	456 ± 132	482 ± 185
PND 120	350 ± 90	369 ± 179	335 ± 110	373 ± 156

[a] Data obtained from Text Tables 12 and 13, page 49, MRID 46814301.
N = 14-16/sex/group

TABLE 10a. Mean (±SD) sub-session motor activity (#movements/10 minute interval) in F_1 rats [a]

Interval	Dietary concentration (ppm)							
	0	20	80	200	0	20	80	200
	Males				Females			
PND 13								
1	11 ± 11	17 ± 20	23 ± 36	15 ± 24	7 ± 10	10 ± 10	19 ± 18	10 ± 13
2	14 ± 23	18 ± 32	18 ± 25	7 ± 12	8 ± 10	9 ± 16	13 ± 13	9 ± 14
3	15 ± 17	12 ± 22	16 ± 17	11 ± 18	10 ± 16	7 ± 10	9 ± 15	7 ± 8
4	9 ± 13	10 ± 18	16 ± 22	11 ± 14	7 ± 11	2 ± 2	12 ± 18	11 ± 21
5	10 ± 19	13 ± 12	14 ± 16	9 ± 14	6 ± 11	6 ± 10	15 ± 17	13 ± 24
6	13 ± 22	22 ± 27	15 ± 19	12 ± 17	4 ± 10	6 ± 14	12 ± 12	11 ± 17
PND 17								
1	55 ± 29	55 ± 43	71 ± 38	58 ± 32	51 ± 37	48 ± 37	58 ± 40	44 ± 30
2	36 ± 26	35 ± 29	40 ± 24	44 ± 30	41 ± 34	40 ± 31	34 ± 26	27 ± 25
3	32 ± 31	21 ± 25	42 ± 33	27 ± 28	25 ± 25	27 ± 21	21 ± 20	36 ± 32
4	17 ± 20	24 ± 29	19 ± 24	19 ± 22	38 ± 39	29 ± 25	28 ± 37	25 ± 30
5	25 ± 32	24 ± 31	25 ± 24	23 ± 25	24 ± 29	14 ± 26	25 ± 34	20 ± 30
6	13 ± 23	27 ± 31	26 ± 33	28 ± 32	35 ± 40	9 ± 21	27 ± 29	18 ± 27
PND 21								
1	111 ± 37	102 ± 25	97 ± 13	100 ± 29	88 ± 26	107 ± 20	88 ± 24	94 ± 25
2	53 ± 29	55 ± 29	59 ± 19	55 ± 36	52 ± 34	50 ± 27	54 ± 23	42 ± 25
3	35 ± 26	36 ± 24	42 ± 22	36 ± 29	54 ± 25	44 ± 19	48 ± 22	39 ± 28
4	32 ± 26	20 ± 26	39 ± 27	20 ± 25	45 ± 29	44 ± 33	35 ± 24	28 ± 23
5	35 ± 34	29 ± 31	30 ± 19	29 ± 31	33 ± 32	33 ± 36	27 ± 25	28 ± 22
6	35 ± 43	20 ± 26	22 ± 18	28 ± 30	40 ± 40	21 ± 31	12 ± 19	30 ± 25
PND 60								
1	106 ± 23	109 ± 24	106 ± 21	112 ± 19	128 ± 24	128 ± 40	130 ± 30	151 ± 31
2	85 ± 19	104 ± 31	97 ± 28	98 ± 24	115 ± 26	107 ± 39	114 ± 31	111 ± 37
3	79 ± 29	108 ± 32	89 ± 30	98 ± 37	112 ± 32	109 ± 52	120 ± 41	117 ± 47
4	83 ± 32	82 ± 39	90 ± 32	79 ± 24	126 ± 36	136 ± 62	119 ± 37	128 ± 47
5	78 ± 28	83 ± 31	82 ± 27	84 ± 33	109 ± 26	109 ± 51	114 ± 40	116 ± 43
6	81 ± 36	72 ± 28	73 ± 31	72 ± 31	105 ± 33	107 ± 48	113 ± 39	107 ± 41
PND 120								
1	92 ± 21	97 ± 25	102 ± 20	106 ± 26	123 ± 34	120 ± 36	105 ± 22	131 ± 45
2	67 ± 18	75 ± 17	75 ± 28	72 ± 19	85 ± 27	88 ± 36	80 ± 28	88 ± 34
3	58 ± 19	59 ± 17	72 ± 32	65 ± 20	92 ± 35	85 ± 41	88 ± 35	98 ± 44
4	59 ± 13	53 ± 22	63 ± 31	59 ± 27	77 ± 28	93 ± 44	82 ± 32	85 ± 40
5	50 ± 22	55 ± 25	53 ± 25	48 ± 22	79 ± 29	81 ± 37	74 ± 35	85 ± 45
6	57 ± 14	61 ± 33	51 ± 31	49 ± 19	86 ± 24	78 ± 36	78 ± 36	93 ± 42

[a] Data obtained from pages 195-204, MRID 46814301.
N = 14-16/sex/group

TABLE 10b. Mean (±SD) sub-session locomotor activity (#movements/10 minute interval) in F_1 rats [a]

Interval	Dietary concentration (ppm)							
	0	20	80	200	0	20	80	200
	Males				Females			
PND 13								
1	2 ± 1	2 ± 3	2 ± 3	1 ± 1	2 ± 2	2 ± 3	2 ± 3	2 ± 3
2	1 ± 2	3 ± 6	2 ± 4	1 ± 2	2 ± 3	1 ± 1	1 ± 2	1 ± 2
3	2 ± 3	2 ± 3	1 ± 2	2 ± 5	2 ± 4	0 ± 1	1 ± 3	2 ± 3
4	1 ± 5	1 ± 4	1 ± 2	1 ± 2	2 ± 3	0 ± 0	1 ± 3	1 ± 1
5	2 ± 4	2 ± 3	2 ± 3	2 ± 3	1 ± 2	1 ± 2	2 ± 4	2 ± 5
6	0 ± 1	5 ± 8	1 ± 2	3 ± 5	0 ± 0	0 ± 1	1 ± 2	1 ± 3
PND 17								
1	14 ± 10	14 ± 13	17 ± 13	14 ± 10	13 ± 13	14 ± 13	15 ± 10	11 ± 9
2	10 ± 6	8 ± 8	10 ± 8	11 ± 9	9 ± 10	10 ± 7	8 ± 8	7 ± 8
3	8 ± 8	6 ± 7	11 ± 10	7 ± 8	7 ± 9	9 ± 9	6 ± 6	9 ± 9
4	4 ± 5	6 ± 7	4 ± 7	3 ± 5	12 ± 13	9 ± 8	6 ± 8	8 ± 12
5	7 ± 10	6 ± 8	7 ± 9	7 ± 8	6 ± 9	5 ± 10	7 ± 9	6 ± 10
6	3 ± 6	7 ± 9	6 ± 9	8 ± 10	10 ± 13	3 ± 8	8 ± 10	4 ± 7
PND 21								
1	39 ± 15	35 ± 13	35 ± 5	34 ± 11	30 ± 10	36 ± 8	33 ± 13	31 ± 11
2	15 ± 8	12 ± 8	17 ± 7	12 ± 9	14 ± 9	13 ± 10	13 ± 8	13 ± 9
3	11 ± 8	9 ± 6	12 ± 10	10 ± 8	15 ± 10	12 ± 8	13 ± 6	11 ± 7
4	10 ± 8	6 ± 8	12 ± 9	5 ± 9	12 ± 9	11 ± 10	10 ± 9	8 ± 7
5	10 ± 10	7 ± 8	10 ± 7	7 ± 7	9 ± 9	8 ± 10	7 ± 8	9 ± 9
6	9 ± 12	4 ± 6	6 ± 6	8 ± 8	10 ± 12	4 ± 6	3 ± 9	9 ± 8
PND 60								
1	75 ± 19	80 ± 19	78 ± 19	76 ± 15	84 ± 13	81 ± 19	86 ± 19	98 ± 35
2	54 ± 18	71 ± 32	70 ± 26	71 ± 23	74 ± 28	66 ± 23	70 ± 19	68 ± 36
3	56 ± 26	74 ± 30	62 ± 25	73 ± 31	75 ± 32	67 ± 36	77 ± 34	76 ± 42
4	57 ± 26	55 ± 32	61 ± 28	56 ± 21	89 ± 35	91 ± 47	79 ± 37	87 ± 43
5	49 ± 23	58 ± 25	59 ± 25	60 ± 25	78 ± 26	65 ± 37	72 ± 35	78 ± 39
6	53 ± 26	46 ± 22	51 ± 26	48 ± 24	70 ± 27	67 ± 37	73 ± 28	75 ± 37
PND 120								
1	67 ± 17	69 ± 17	77 ± 17	76 ± 19	82 ± 18	84 ± 28	74 ± 20	87 ± 30
2	43 ± 12	49 ± 13	50 ± 19	50 ± 14	50 ± 13	61 ± 31	55 ± 17	54 ± 27
3	35 ± 15	35 ± 12	45 ± 20	42 ± 16	59 ± 27	56 ± 33	60 ± 24	59 ± 29
4	40 ± 10	31 ± 19	41 ± 24	39 ± 17	49 ± 20	62 ± 39	52 ± 23	54 ± 29
5	31 ± 12	35 ± 17	34 ± 19	29 ± 14	51 ± 23	53 ± 34	44 ± 21	56 ± 31
6	39 ± 12	37 ± 20	34 ± 21	34 ± 15	59 ± 22	53 ± 33	51 ± 26	62 ± 30

[a] Data obtained from pages 206-215, MRID 46814301.
N = 14-16/sex/group

d. <u>Auditory startle reflex habituation</u>: The overall amplitude and latency data are presented in Table 11. Interval amplitude and latency data are included in Tables 11a and 11b, respectively. Startle amplitude, latency and habituation were not affected by treatment. The amplitude of the startle response increased with age in both sexes. Habituation was apparent in control and treated animals on PND 60 but not on PND 22.

TABLE 11. Mean (±S.D.) auditory startle peak amplitude and latency to peak in F_1 male and female rats [a]				
Test Day	**Dietary concentration (ppm)**			
	0	**20**	**80**	**200**
Males				
PND 22				
Number of animals	16	16	16	16
Body weight (g)	52	53	52	48
Peak amplitude (g)	28 ± 9	24 ± 10	27 ± 11	26 ± 10
Latency to peak (msec)	37 ± 3	38 ± 3	38 ± 4	38 ± 5
PND 60				
Number of animals	16	16	16	16
Body weight (g)	273	280	270	272
Peak amplitude (g)	157 ± 128	112 ± 79	148 ± 100	143 ± 63
Latency to peak (msec)	40 ± 4	38 ± 5	40 ± 3	38 ± 3
Females				
PND 22				
Number of animals	16	16	16	16
Body weight (g)	52	51	48	48
Peak amplitude (g)	27 ± 12	27 ± 8	27 ± 13	27 ± 8
Latency to peak (msec)	40 ± 6	39 ± 5	39 ± 5	38 ± 5
PND 60				
Number of animals	16	16	16	16
Body weight (g)	174	179	168	171
Peak amplitude (g)	73 ± 37	80 ± 42	102 ± 52	92 ± 56
Latency to peak (msec)	42 ± 5	41 ± 2	40 ± 3	41 ± 3

[a] Data obtained from Text Tables 14 and 15, pages 51-52, MRID 46814301.

TABLE 11a. Mean (±S.D.) interval auditory startle peak amplitude (g) in F_1 male and female rats [a]				
Test Day	Dietary concentration (ppm)			
	0	20	80	200
Males				
PND 22				
Block 1	28 ± 9	26 ± 11	29 ± 14	27 ± 12
Block 2	29 ± 10	27 ± 11	27 ± 13	28 ± 10
Block 3	28 ± 9	25 ± 11	28 ± 14	28 ± 13
Block 4	27 ± 12	23 ± 12	27 ± 10	25 ± 12
Block 5	26 ± 9	19 ± 9	24 ± 10	22 ± 9
PND 60				
Block 1	175 ± 129	113 ± 72	171 ± 109	164 ± 77
Block 2	183 ± 153	138 ± 107	189 ± 142	186 ± 106
Block 3	198 ± 156	124 ± 96	155 ± 113	167 ± 84
Block 4	142 ± 117	115 ± 83	120 ± 92	123 ± 55
Block 5	116 ± 99	111 ± 57	127 ± 95	91 ± 40
Females				
PND 22				
Block 1	32±15	29±10	28±19	33±14
Block 2	29±15	28±9	30±15	30±13
Block 3	27±13	30±10	27±13	26±8
Block 4	26±10	26±10	26±12	26±8
Block 5	22±11	23±8	25±13	21±6
PND 60				
Block 1	86±53	92±41	120±68	114±65
Block 2	77±49	98±68	129±74	102±66
Block 3	70±37	93±69	111±59	95±64
Block 4	71±49	61±39	85±49	77±52
Block 5	59±30	54±31	64±35	71±56

[a] Data obtained from Text Table 14, page 51, MRID 46814301.

TABLE 11b. Mean (±S.D.) interval auditory startle latency to peak (msec) in F_1 male and female rats [a]				
Test Day	Dietary concentration (ppm)			
	0	20	80	200
Males				
PND 22				
Block 1	39±5	42±8	39±5	39±5
Block 2	37±4	37±4	39±7	38±7
Block 3	37±4	38±4	37±5	38±7
Block 4	37±4	36±3	37±4	37±4
Block 5	38±5	38±5	38±7	38±6
PND 60				
Block 1	41±4	39±7	42±3	41±4
Block 2	41±3	42±7	40±4	36±10
Block 3	40±3	39±3	39±4	37±3
Block 4	40±5	37±3	39±3	39±6
Block 5	41±4	39±5	39±4	39±4
Females				
PND 22				
Block 1	42±8	41±7	40±5	40±7
Block 2	40±7	39±7	40±6	37±6
Block 3	38±6	38±7	39±8	38±7
Block 4	40±8	38±6	38±7	38±7
Block 5	40±5	37±5	39±7	39±7
PND 60				
Block 1	44±5	43±4	41±3	42±4
Block 2	43±5	41±4	39±3	40±3
Block 3	40±5	40±4	39±4	41±6
Block 4	43±7	41±5	40±6	42±6
Block 5	42±6	42±5	38±4	41±5

[a] Data obtained from Text Table 15, page 52, MRID 46814301.

d) **Learning and memory testing:** Results from passive avoidance testing are given in Table 12. On the first test, acquisition was evident in control males and females as a marked increase in the latency to cross for the second trial, compared to the first trial. On the second test, retention was evident in control males and females as a protracted delay to cross within the 180-second time limit of the first trial, compared to the first trial on the first test day. No treatment-related effects were observed.

Results from the water maze are given in Table 13. A statistical difference from control during acquisition in mid- and high-dose females involving a significant decrease in the number of errors during the first trial (0.6 and 0.4, respectively, vs. an average of 1.6 errors for controls) was observed on PND 60±2. This finding was not considered treatment-related since the change was not observed in males and the values were within the historical controls (average 0.3 to 1.3 errors), whereas the number of errors for controls was above the range for the historical control.

TABLE 12. Passive avoidance performance in PND 22 offspring (mean ± S.D.) [a]

Session/Parameter		Dietary concentration (ppm)			
		Control	20	80	200
Males					
Session 1 (PND 22)[b]	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	2.9±0.3	3.2±0.8	3.1±0.6	2.9±0.3
	Latency trial 1 (sec)	52.9±50.0	31.8±29.2	49.7±50.9	47.0±42.3
	Latency trial 2 (sec)	180.0±0.0	169.6±41.7	180.0±0.0	180.0±0.0
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	1 (6%)	0 (0%)	1 (6%)	1 (6%)
Session 2 (PND 29)[b]	Number of Animals Tested	15	16	15	15
	Number of Animals Included in Analysis	15	16	15	15
	Trials to criterion	2.3±0.7	2.4±0.8	2.3±0.7	2.2±0.4
	Latency trial 1 (sec)	180.0±0.0	177.8±8.7	178.4±6.1	160.4±50.8
	Latency trial 2 (sec)	169.2±29.7	166.8±40.3	173.6±17.0	180.0±0.0
Females					
Session 1 (PND 22)[b]	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	3.0±0.0	3.3±1.0	3.2±0.4	3.3±0.7
	Latency trial 1 (sec)	25.5±24.7	38.7±39.2	47.4±42.1	23.5±17.2
	Latency trial 2 (sec)	180.0±0.0	180.0±0.0	172.4±21.3	180.0±0.0
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Failed to Cross During Learning Phase	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Session 2 (PND 29)[b]	Number of Animals Tested	16	16	16	16
	Number of Animals Included in Analysis	16	16	16	16
	Trials to criterion	2.3±0.6	2.3±0.5	2.1±0.3	2.4±0.7
	Latency trial 1 (sec)	162.6±47.7	151.6±50.7	175.9±13.2	166.2±34.2
	Latency trial 2 (sec)	171.0±36.0	180.0±0.0	180.0±0.0	174.2±19.8

[a] Data extracted from Text Table 16, page 54, MRID 46814301.

[b] Session 1 = learning phase; Session 2 = retention phase.

Trials to Criterion = Mean # Trials per Group ± S.D.
Latency to Trial 1 = Mean Session 1 duration (seconds) per Group ± S.D.
Latency to Trial 2 = Mean Session 2 duration (seconds) per Group ± S.D.
Failed to Meet Criterion = Number of Animals that received the shock but did not demonstrate acquisition.
Failed to Cross = Number of Animals that never received the shock.

Table 13. Water maze performance data (mean ± S.D.) [a]

Session/Parameter		Dietary concentration (ppm)			
		Control	20	80	200
Males					
Session 1 (PND 60±2) [b]	Number of Animals	16	16	16	16
	Trials to Criterion (Mean±S.D.)	6.9±2.4	6.8±2.4	6.5±2.0	7.9±2.1
	Trial 1 - Errors (Mean±S.D.)	0.6±0.9	1.0±1.2	0.6±0.6	0.8±0.9
	Trial 1 - Duration (sec) (Mean±S.D.)	18.8±15.1	21.9±15.0	12.1±5.9	19.3±18.4
	Trial 2 - Errors (Mean±S.D.)	0.6±0.6	0.3±0.7	0.6±1.0	0.9±0.8
	Trial 2 - Duration (sec) (Mean±S.D.)	12.6±9.9	17.6±15.6	16.0±15.9	20.3±15.1
	Failed to Meet Criterion	1 (6%)	0 (0%)	0 (0%)	0 (0%)
Session 2 (PND 67±2) [b]	Number of Animals	15	16	16	16
	Trials to Criterion (Mean±S.D.)	5.2±0.4	6.0±1.6	5.6±1.7	5.6±1.1
	Trial 1 - Errors (Mean±S.D.)	0.2±0.4	0.4±0.9	0.4±0.7	0.6±1.5
	Trial 1 - Duration (sec) (Mean±S.D.)	7.5±4.3	10.6±11.4	8.5±7.2	11.2±14.7
	Trial 2 - Errors (Mean±S.D.)	0.0±0.0	0.0±0.0	0.2±0.8	0.1±0.3
	Trial 2 - Duration (sec) (Mean±S.D.)	4.7±1.5	4.3±1.3	5.3±6.2	4.2±1.6
Females					
Session 1 (PND 60±2) [b]	Number of Animals	16	16	16	16
	Trials to Criterion (Mean±S.D.)	8.1±2.1	7.4±2.3	7.2±2.0	8.1±2.8
	Trial 1 - Errors (Mean±S.D.)	1.6±1.5	0.8±0.9	0.6*±0.6	0.4*±0.9
	Trial 1 - Duration (sec) (Mean±S.D.)	23.3±17.0	16.6±9.3	12.8±6.9	13.9±13.4
	Trial 2 - Errors (Mean±S.D.)	1.0±1.2	0.6±0.7	0.9±1.2	0.6±0.7
	Trial 2 - Duration (sec) (Mean±S.D.)	17.9±15.0	11.8±5.2	14.0±14.2	10.4±5.3
	Failed to Meet Criterion	0 (0%)	0 (0%)	0 (0%)	1 (6%)
Session 2 (PND 67±2) [b]	Number of Animals	16	16	16	15
	Trials to Criterion (Mean±S.D.)	7.6±3.2	6.5±2.7	7.2±3.6	8.7±3.5
	Trial 1 - Errors (Mean±S.D.)	0.6±0.8	0.4±1.3	0.3±0.6	0.1±0.4
	Trial 1 - Duration (sec) (Mean±S.D.)	9.9±7.2	9.5±11.1	6.6±4.4	6.3±3.7
	Trial 2 - Errors (Mean±S.D.)	0.3±0.6	0.3±0.7	0.1±0.3	0.7±1.0
	Trial 2 - Duration (sec) (Mean±S.D.)	5.3±3.8	5.2±3.3	4.1±1.7	7.6±5.2

[a] Data obtained from Text Table 17, page 56, MRID 46814301.

[b] Session 1 = learning phase; Session 2 = retention phase.

Values for rats who failed to learn during session 1 were not included in means for session 2.

Values are mean ± standard deviation

* Statistically different from control, p#0.05

5. **Ophthalmology**: No treatment-related effects were observed. Findings, including corneal opacity, retinal degeneration, myosis, iritis and corneal edema, were reported in control and treated animals.

6. **Postmortem results:**

   a. **Brain weight:** Mean brain weight data are presented Table 14. Absolute brain weight in males and females was not affected by treatment on PND 21. On PND 75, fixed brain weight in perfused high-dose females was significantly decreased (7%).

TABLE 14. Mean (±SD) brain weight data [a]				
Parameter	**Dietary concentration (ppm in diet)**			
	Control	**20**	**80**	**200**
Males				
Day 21 (Perfused)				
Terminal Body Weight (g)	48.3±6.5	49.3±3.7	48.9±6.1	44.8±2.9
Brain, Fixed (g)	1.42±0.07	1.40±0.05	1.41±0.06	1.42±0.05
PND 75 (Termination - Perfused)				
Terminal Body Weight (g)	326.3±22.2	323.2±16.6	320.5±31.3	318.6±19.8
Brain, Fixed (g)	1.86±0.07	1.87±0.12	1.87±0.07	1.89±0.11
PND 75 (Termination - Non-Perfused)				
Terminal Body Weight (g)	320.5±24.1	328.7±16.5	316.6±26.6	325.9±14.3
Brain, Fresh (g)	1.93±0.08	1.94±0.13	1.88±0.11	1.95±0.09
Females				
Day 21 (Perfused)				
Terminal Body Weight (g)	48.9±3.4	45.2±5.3	45.3±5.0	45.2±4.5
Brain, Fixed (g)	1.39±0.05	1.35±0.05	1.36±0.06	1.35±0.07
PND 75 (Termination - Perfused)				
Terminal Body Weight (g)	193.9±13.1	195.0±11.6	192.6±11.4	189.1±12.2
Brain, Fixed (g)	1.79±0.07	1.79±0.08	1.75±0.06	1.67*±0.09 (↓7)
PND 75 (Termination - Non-Perfused)				
Terminal Body Weight (g)	200.00±16.4	199.0±15.9	188.5±9.8	191.4±14.6
Brain, Fresh (g)	1.82±0.08	1.77±0.12	1.77±0.09	1.76±0.10

[a] Data obtained from Text Table 18, page 59, MRID 46814301.
* Statistically different from control, p#0.05
N = 10

b) Neuropathology:

1. **Macroscopic examination:** No treatment-related lesions were observed. Incidental lesions included: a pitted zone in the cerebrum of one perfused female at 20 ppm on PND 21; skin crusty zone in one non-perfused male at 80 ppm at the terminal necropsy; and dilated renal pelvis in the kidney of a control non-perfused male and a non-perfused female at 20 ppm at the terminal necropsy.

2. **Microscopic examination:** Selected microscopic data are presented in Table 15. Degeneration of several nerve fibers was observed at low incidence in treated and control animals at the terminal necropsy. Severity was graded minimal for all findings of nerve fiber degeneration. Optic nerve hemorrhaging was seen in treated males and females. This finding was considered incidental and attributed to removal of the eye from the skull and not associated with treatment.

TABLE 15. Histopathology findings at terminal necropsy (PND 75) [number affected/number examined (mean severity score)] [a]

Parameter	Dietary concentration (ppm)			
	0	20	80	200
Males				
Nerve, sciatic, right – degeneration	0/10	--	--	2/10 (1.0)
Nerve, sciatic, left – degeneration	0/10	--	--	0/10
Nerve, sural, right – degeneration	0/10	--	--	0/10
Nerve, sural, left – degeneration	0/10	--	--	0/10
Nerve, tibial, right – degeneration	2/10 (1.0)	--	--	0/10
Nerve, tibial, left – degeneration	1/10 (1.0)	--	--	1/10 (1.0)
Optic nerve				
Hemorrhage	0/10	--	--	1/10 (4.0)
Vacuolization	2/10 (1.5)	--	--	0/10
Spinal nerve root – degeneration	0/10	--	--	2/10 (1.0)
Females				
Nerve, sciatic, right – degeneration	1/10 (1.0)	--	--	1/10 (1.0)
Nerve, sciatic, left – degeneration	0/10	--	--	1/10 (1.0)
Nerve, sural, right – degeneration	0/10	--	--	0/10
Nerve, sural, left – degeneration	0/10	--	--	1/10 (1.0)
Nerve, tibial, right – degeneration	1/10 (1.0)	--	--	1/10 (1.0)
Nerve, tibial, left – degeneration	1/10 (1.0)	--	--	0/10
Optic nerve				
Hemorrhage	0/10	--	--	2/10 (3.5)
Vacuolization	0/10	--	--	0/10
Spinal nerve root – degeneration	1/10 (1.0)	--	--	1/10 (1.0)

[a] Data obtained from pages 873-876, MRID 46814301.
-- = Not examined.

2. **Brain Morphometry**: Morphometric data are included in Tables 16 (PND 21) and 17 (PND 75). On PND 21, a statistical increase in the cerebrum length in males at 80 ppm was not considered treatment-related since there was no dose response. On PND 75, a statistically significant increase in the hippocampus thickness was observed in males at 200 ppm. The value was within the historical control range (1.57-1.74 mm), while the control value was decreased in relation to the historical control.

TABLE 16. Mean (±S.D.) morphometric measurements (mm) in perfused F_1 rats on PND 21[a]

Parameter	Dietary concentration (ppm)			
	Control	20	80	200
Males				
Ant/Post Cerebrum Length (mm)	13.30±0.15	13.48±0.18	13.69*±0.22	13.38±0.29
Ant/Post Cerebellum (mm)	7.07±0.34	7.02±0.47	6.97±0.25	7.29±0.24
Frontal Cortex (mm)	1.80±0.03	--	--	1.81±0.01
Parietal Cortex (mm)	1.92±0.03	--	--	2.0±0.01
Caudate Putamen (mm)	2.98±0.02	--	--	2.99±0.04 (n=9)
Hippocampal Gyrus (mm)	1.75±0.02	--	--	1.80±0.00
Cerebellum (mm)	4.19±0.11	--	--	4.34±0.08
Females				
Ant/Post Cerebrum Length (mm)	13.35±0.16	13.36±0.34	13.50±0.25	13.23±0.29
Ant/Post Cerebellum (mm)	7.20±0.30	6.87±0.24	6.78*±0.29	6.98±0.44
Frontal Cortex (mm)	1.73±0.02	--	--	1.77±0.01
Parietal Cortex (mm)	1.91±0.02	--	--	1.88±0.01
Caudate Putamen (mm)	2.86±0.03	--	--	2.92±0.03
Hippocampal Gyrus (mm)	1.66±0.01	--	--	1.67±0.00
Cerebellum (mm)	4.32±0.08	--	--	4.23±0.08

[a] Data obtained from Text Table 19, pages 61-62, MRID 46814301.

-- = not evaluated

N = 10, except where noted.

* Statistically different from control, p#0.05

TABLE 17. Mean (±S.D.) morphometric measurements (mm) in perfused F_1 rats on PND 75[a]				
Parameter	**Dietary concentration (ppm)**			
	Control	**20**	**80**	**200**
Males				
Ant/Post Cerebrum Length (mm)	14.58±0.29	14.57±0.41	14.47±0.42	14.63±0.39
Ant/Post Cerebellum (mm)	7.89±0.51	7.83±0.57	7.72±0.43	7.85±0.37
Frontal Cortex (mm)	1.66±0.13	-	-	1.68±0.01
Parietal Cortex (mm)	1.78±0.01	-	-	1.83±0.01
Caudate Putamen (mm)	3.20±0.02	-	-	3.29±0.02
Hippocampal Gyrus (mm)	1.55±0.03	-	-	1.73*±0.02
Cerebellum (mm)	4.96±0.14	-	-	4.97±0.27
Females				
Ant/Post Cerebrum Length (mm)	13.96±0.33	14.15±0.37	14.33±0.28	14.00±0.43
Ant/Post Cerebellum (mm)	7.98±0.42	7.90±0.42	7.88±0.40	7.64±0.46
Frontal Cortex (mm)	1.74±0.01	-	-	1.66±0.02
Parietal Cortex (mm)	1.81±0.00	-	-	1.76±0.02
Caudate Putamen (mm)	3.31±0.03	-	-	3.17±0.02
Hippocampal Gyrus (mm)	1.60±0.04	-	-	1.63±0.01
Cerebellum (mm)	4.98±0.44	-	-	4.83±0.23

[a] Data obtained from Text Table 19, pages 61-62, MRID 46814301.

– = not evaluated

N = 10

* Statistically different from control, p#0.05.

III. DISCUSSION and CONCLUSIONS:

A. INVESTIGATORS CONCLUSIONS:

The study author concluded there was no effect on reproduction parameters at any dietary level. Significantly decreased body weight, body weight gain, and food consumption during gestation and lactation were observed in maternal animals at 200 ppm. Significantly decreased pre- and post-weaning body weight and body weight gain, vocalizations with handling in males on PND 4 and a delay in balanopreputial separation were reported in offspring at 200 ppm.

B. REVIEWER COMMENTS:

Administration of technical grade deltamethrin in the diet at a concentration of 200 ppm (16.1 mg/kg/day) produced no deaths or clinical signs of toxicity in parental females. Decreased body weight gain, corresponding with lower food consumption, generally during the second week of gestation resulted in significantly reduced absolute body weight at 200 ppm. The lower body weight for this group persisted into the first week of lactation with recovery apparent thereafter. No treatment-related effects on reproduction were reported.

Litter size and viability were not affected by treatment. The sex ratio (% males) was slightly lower at 200 ppm, but no dose-response was observed. No clinical signs of toxicity were

observed in offspring. Body weight in offspring during the pre-weaning and post-weaning periods was decreased in males and females. The onset of balanopreputial separation was delayed in males at 200 ppm and attributed to the lower body weight of these animals compared with the controls. While the onset of vaginal opening was slightly delayed in females at 200 ppm, the day of reaching criterion was within the historical control range and there was no dose-response effect. Therefore, the change is not considered treatment-related.

During the FOB on PND 4, an increased number of males at 200 ppm minimally resisted handling with vocalizations. Motor activity, auditory startle response and learning and memory testing were not affected by treatment. Fixed brain weight at the terminal necropsy was decreased in females at 200 ppm; although fresh brain weight of females was not similarly affected, the finding is considered treatment-related and can not be discounted. Microscopically, degeneration of nerve fibers is a common finding, the lesions occurred at low incidence in controls and high-dose animals, and the severity was minimal. Morphometric measurements were unaffected by treatment at the PND 21 necropsy. Optic nerve hemorrhaging was seen in treated males and females. This finding was considered incidental and attributed to removal of the eye from the skull and not associated with treatment. Hippocampus thickness was decreased in males at 200 ppm during the terminal necropsy; the change is not considered treatment-related since the value was within the historical control range and no other morphometric effects were observed.

The maternal LOAEL is 200 ppm (16.1 mg/kg/day) based on decreased body weight, body weight gain and food consumption. The maternal NOAEL is 80 ppm (6.78 mg/kg/day).

The offspring LOAEL is 200 ppm (16.1 mg/kg/day) based on decreased body weight and body weight gain during pre-weaning and post-weaning in males and females, increased incidence of vocalizations (males) during FOB handling, and decreased fixed brain weight in females at the terminal necropsy. The offspring NOAEL is 80 ppm (6.78 mg/kg/day).

C. STUDY DEFICIENCIES:

Brain assays for deltamethrin were performed, but the results were not reported.

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R157454

Chemical: Deltamethrin

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